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Serum β-Glucuronidase Activity in a Population of Ex-Coalminers

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Background: The aim of this study was to investigate whether BGD activity is of additional value in the assessment of pulmonary inflammation caused by coal dust exposure.
Design and methods: Ex-coalminers were included in this study. Forty-eight healthy male subjects, without a relevant medical history, were used as controls.
Results: In ex-coalminers serum BGD activity was higher compared to the control group. Moreover, ex-coalminers with a normal chest radiograph and normal serum LDH demonstrated elevated serum BGD compared to the control group. However, no relation was found in the total group of ex-coalminers between serum BGD activity and pulmonary function parameters.
Conclusions: Our study adds *in vivo* human evidence to the already existing animal data that BGD is a potential biomarker useful in monitoring pulmonary inflammation caused by coal dust exposure.

KEY WORDS: beta-glucuronidase; BGD; coal dust; ex-coalminers.

Introduction

Coal workers' pneumoconiosis (CWP) is a chronic inflammatory and fibrotic lung disease caused by prolonged exposure to coal dust (1–5). Coal dust exposure results in activation of phagocytic alveolar macrophages (AMs) followed by an acute inflammatory response, damage to the respiratory epithelial cells and interstitial matrix (6). The cytotoxic activity of coal dust was found to be related to damage of cellular membranes, impaired host defence mechanisms and release of reactive oxygen compounds, hydrolytic enzymes, and other inflammatory mediators. These processes may participate in the development of chronic lung inflammation and fibrosis (4,5,7). It is now evident that CWP can become apparent or progress further because of the persistent cytotoxic effect of silica, an important component of coal dust, even after cessation of exposure (2).

To characterize the nature and extent of coal dust induced airway injury there is a need for biomarkers (8,9). Biomarkers include markers of exposure to external influences, markers of susceptibility to develop a specific disease and markers of pathophysiological changes related to the disease (9,10). Biomarkers of exposure are important, in particular, if environmental or biological factors are studied, *e.g.,* in case of occupational exposure. However, they are of less importance in monitoring disease activity. A biomarker of susceptibility may reveal why some coalminers are at risk of developing CWP and others are not. The potential of many cell mediators as biomarkers, *e.g.*, Clara cell protein (CC-16) (11), surfactant associated protein (12), antioxidants and several cytokines (13,14) has been raised many times, but pathognomic criteria or "a golden standard" for monitoring the effect of coal dust exposure does not exist. Therefore, searching for other parameters useful to monitor exposure effects is still of benefit.

Previously, the authors demonstrated an increase in lactate dehydrogenase (LDH) and changes of the LDH isoenzyme pattern in serum of ex-coalminers (15). Phagocytic cells, such as AMs and polymorphonuclear neutrophils (PMNs), help to clear the lung of inhaled particles, including inorganic dusts. Dust particles directly or indirectly stimulate these cells to release the earlier mentioned mediators. The increase of lysosomal enzymes appeared to be useful in monitoring phagocytic activity or lysis of phagocytic cells (16,17). Beta-glucuronidase (BGD) was found to be increased in bronchoalveolar lavage fluid (BALF) of animals after instillation of respirable pneumotoxicants (16–21). However, the only human study using BGD as a marker of inflammation did not find an increase of BGD in BALF after a short period of ozone exposure (22). In contrast, Thompson *et al.*(23) found an increase of serum angiotensin-

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TABLE 1
Characteristics and Pulmonary Function Parameters of the Studied Population of Ex-Coalminers and Available Data of the Non-Exposed Control Group

	Ex-coalminers (n = 191)	Controls (n = 48)
Age (years)	72 ± 6 (70.6–72.6)	58 ± 13 (54.1–61.5)**
Weight (kg)	76 ± 13 (74.0–77.6)	81 ± 10 (78.4–84.4)*
Height (cm)	170 ± 7 (168.9–171.0)	182 ± 12 (178.3–185.2)**
Years underground	26 ± 7 (24.5–27.0)	0**
Smoking (pack years)[a]	30 ± 16 (27.4–33.0)	24 ± 17 (16.8–30.5)**
FEV_1 of norm (%)	69 ± 22 (66.1–72.5)	Not done
FVC of norm (%)	95 ± 19 (92.1–97.6) n = 178	Not done
D_{CO} (%)	69 ± 20 (66.0–71.9)	Not done

Data are expressed as mean ± SD and range in 95% confidence intervals in parentheses. Pulmonary function tests are expressed in body temperature and pressure, saturated with water vapor (BTPS): FEV_1 = forced expiratory volume in one second; FVC = forced expiratory volume; D_{CO} = diffusion capacity measured by single breath method.

[a]Pack years of smokers.

*$p < 0.005$ and **$p < 0.0001$ controls versus population of ex-coalminers.

converting enzyme (ACE) and lysozyme, other lysosomal enzymes, after coal dust or silica exposure.

Consistent with the concept that inhalation of pneumotoxicants results in the presence of activated inflammatory cells, we hypothesized that BGD could be considered as a biochemical marker of the inflammatory response caused by coal dust. Therefore, the aim of this study was to examine the serum BGD activity in ex-coalminers compared to non-exposed individuals. Furthermore, the relationship between BGD activity in serum with other clinical parameters was studied. Particularly, the question, whether the BGD increase was linked with a LDH increase or appeared independently, was evaluated.

Methods

PATIENTS

The study was performed within a population of ex-coalminers (n = 191, all males). These ex-coalminers were invited for a medical check-up. The ex-coalminers were not selected, nor were actual complaints reason for their visit to the outdoor patient department. All had a history of coal dust exposure, more than 20 years ago. Their medical history revealed no other relevant pulmonary disorders. The majority of the ex-coalminers (n = 134) were smokers, with a smoking history of many years. Only 14 ex-coalminers were non-smokers, while 43 had unknown smoking status (for personal characteristics, see Table 1). The chest radiograph was classified as normal in 49 ex-coalminers. The chest radiograph was classified as abnormal (n = 142) showing abnormalities varying between few nodules, normal lung markings visible and numerous opacities, and normal markings totally obscured.

A group of 48 healthy control subjects, all male (age 58 ± 13 years, 15 smokers and 33 non-smokers)—without a relevant medical history—was used to assess reference values of serum BGD, LDH activities and its isoenzyme pattern, total protein, albumin, urea, creatinine, gamma-glutamyl transferase (GGT), alanine amino transferase (ALT), and creatine kinase (CK).

PULMONARY FUNCTION TESTS

Pulmonary function tests were assessed. Forced expiratory volume capacity (FVC) and forced expiratory volume in one second (FEV_1) were determined using a pneumotachograph (Jaeger, Masterlab, Wuerzburg, Germany). Diffusion capacity (D_{CO}) was obtained by the single breath method and corrected for hemoglobin (Jaeger, Masterlab). The reference values for each subject, based on sex, age, and height, were obtained from standard formula (24). Data were expressed as percentages of the reference values.

LABORATORY TESTS

Blood samples were taken and serum was obtained after routine centrifugation (12 min, 2000 *g*). Serum was stored frozen at −70° C until actual measurement. In the authors' laboratory, the activity of BGD (serum 50 μl) was measured at 37° C, using p-nitrophenyl-β-D-glucuronide (4.68 mM) in 85 mM acetate buffer of pH 4.5, as a substrate (50 μl). The product of the enzymatic hydrolysis is p-nitrophenol, which has a strong yellow color in basic solutions due to the absorbance of light at a wavelength of 405 nm. The assay was run in an acetate buffer (50 μl), pH 4.5, and the incubations were stopped at 30 min by the addition of a strong base (0.5 M sodium hydroxide, 150 μl) to develop the color. The assay was run on an automatic plate reader (Cambridge 7520 Microplate Reader, Cambridge Technology, Inc, Watertown, MA, USA).

The LDH activity was measured at 37° C by an enzymatic rate method, using pyruvate as a sub-

TABLE 2
Laboratory Data of the Studied Population of Ex-Coalminers and Non-Exposed Controls

	Ex-coalminers (n = 191)	Controls (n = 48)
Albumin (g/L)	39 ± 3 (38.1–39.1)	44 ± 2 (43.0–44.3)**
Creatinine (mmol/L)	98 ± 21 (94.6–100.6)	97 ± 18 (92.8–103.2)
ALT (U/L)	19 ± 10 (18.2–21.4)	21 ± 8 (18.6–23.2)
GGT (U/L)	29 ± 28 (25.3–33.2)	25 ± 19 (20.1–30.8)
CK (U/L)	93 ± 52 (85.3–100.6)	143 ± 50 (130.1–159.7)†
BGD (U/L)	1.008 ± 0.784 (0.890–1.140)	0.416 ± 0.541 (0.259–0.573)‡
LDH (U/L)	633 ± 247 (597.3–667.7)	359 ± 50 (365.9–377.0)*

Data are expressed as mean ± SD and range in 95% confidence intervals in parentheses: ALT = alanine amino transferase; GGT = gamma-glutamyl transferase; CK = creatine kinase; BGD = β-glucuronidase.

ANOVA (corrected for age): $^{*}F(1,236) = 30.24$, $p < 0.001$, $^{**}F(1,236) = 36.31$, $p < 0.001$, $^{\dagger}F(1,236) = 18.93$, $p < 0.001$ and $^{\ddagger}F(1,236) = 6.1$, $p < 0.02$ versus population of ex-coalminers.

strate. The test was performed on a Beckman Synchron CX-7 system with Beckman reagents (testkit 442660, Beckman Instruments Inc, Mijdrecht, The Netherlands) and was optimized according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (DGKC-recommendations) (25). The system monitors the reduction of pyruvate to L-lactate with the concurrent oxidation of β-nicotinamide adenine dinucleotide (NADH; reduced form) at 340 nm. The change in absorbance at 340 nm, caused by the disappearance of NADH is measured over a fixed time interval and is directly proportional to the LDH activity. LDH activity is expressed in micromoles of substrate (pyruvate) converted per minute (U), per litre serum at 37° C. The measuring range is 10–1800 U/L, for concentrations of 1800–3800 U/L the samples were automatically diluted with saline and re-analyzed and for higher concentrations manual dilution was required. For the determination of the LDH isoenzymes the Beckman Paragon Lactate Dehydrogenase Electrophorese's Kit was used (testkit 655940). Electrophoresis and scanning of the gels were performed with the Beckman Appraise System (Beckman Instruments Inc.).

Serum samples were also analyzed for urea, total protein, albumin, GGT, ALT and CK and were determined on a Synchron CX-7 analyzer (Beckman Instruments Inc., Brea, CA, USA), using testkits from Beckman Instruments Inc.

STATISTICAL METHODS

The significance of differences concerning personal characteristics, laboratory and pulmonary function parameters was tested using Student's t-test for continuous data and χ^2 tests for categorical data. For comparing the group of ex-coalminers with the healthy control group, with respect to laboratory and pulmonary function parameters, a Student's t-test or ANCOVA (age as covariate) was used. Mann-Whitney U-test was used to compare ex-coalminers with normal and ex-coalminers with abnormal chest radiograph. Pearson correlation coefficients were used to test a relation, between serum LDH and BGD activity on the one hand and other laboratory parameters and the performed pulmonary function tests on the other. In addition, Pearson correlation was also used to assess the relationship between serum BGD and LDH. To analyse the association of BGD and LDH with other parameters, multiple regression analysis (stepwise) was performed using serum BGD and serum LDH activity as the dependent variables. For not normally distributed variables log transformations were done. Personal characteristics were entered blockwise before entering laboratory and pulmonary function parameters. A p value of less than 0.05 was considered to be significant. All analyses were performed using the Statistical Package for Social Science (SPSS).

Results

The characteristics of the studied population of ex-coalminers (n = 191), are summarized in Table 1. The laboratory data of the studied group of ex-coalminers, as well as reference values obtained from the healthy subjects, are presented in Table 2. Serum BGD activity (1.008 ± 0.784 U/L; $F(1,236) = 6.1$, $p < 0.02$, Fig. 1) and serum LDH activity (633 ± 247 U/L; $F(1,236) = 30.24$, $p < 0.001$) appeared to be elevated in the group of ex-coalminers. In the population of ex-coalminers only a moderate correlation between the serum BGD and LDH activity ($r = 0.17$, $p < 0.02$), as well as the percentage of LDH_3 ($r = 0.17$, $p < 0.02$) was found. No correlation was found with the other LDH isoenzymes. Serum BGD and LDH activity did not differ between smokers and non-smokers in the group of ex-coalminers, or in the control group. Furthermore, no relation was demonstrated between serum BGD activity and the pulmonary function parameters given in Table 1. Only a moderate negative correlation between the serum BGD activity and the FVC ($r = -0.15$, $p < 0.05$) was found.

In the group of ex-coalminers with a normal chest radiograph (n = 49), the serum BGD (0.809 ± 0.510 U/L; $F(1,76) = 4.76$, $p < 0.05$) and serum LDH

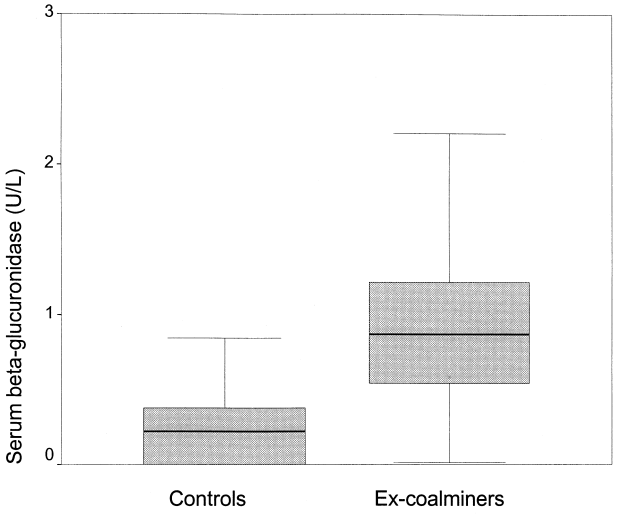

Figure 1 — Box-plot of serum β-glucuronidase activity in the group of ex-coalminers and in healthy control subjects. Boxes indicate interquartile range, the horizontal line indicates the median; ex-coalminers versus controls: $p < 0.02$.

activity (659 ± 233 U/L; $F(1,78) = 18.25$, $p < 0.001$) were significantly increased compared to the control group (Table 2). When comparing the group of ex-coalminers with a normal chest radiograph and the group with an abnormal chest radiograph ($n = 142$), no statistically significant differences were found in pulmonary function parameters, except for the Dco ($U = 914.5$, $p < 0.02$), which was lower in the group with an abnormal chest radiograph. In a group with normal serum LDH activity ($n = 39$) the serum BGD activity (0.860 ± 0.548 U/L; $F(1,84) = 6.60$, $p < 0.05$) was also elevated compared to the control group.

To further analyze the association between serum BGD, LDH activity and pulmonary function and laboratory tests, multiple regression analysis was performed using BGD and LDH as dependent variables. The personal characteristics were forced blockwise into the equation. Within each block the variables were entered stepwise. After having controlled for these variables, the pulmonary function and laboratory variables were entered into the equation. No predicting factor was found in the control group for the serum BGD activity. In the total population of ex-coalminers only the FVC ($\beta = -0.28$, $R^2 = 7.7\%$; $F(1,90) = 7.5$, $p < 0.01$) explained a proportion of the variance in BGD. No predicting variables for the dependent variable LDH were found in the total population of ex-coalminers.

Discussion

To the best of our knowledge, this study is the first to describe a significant increase in serum BGD activity in a group of ex-coalminers compared to a non-exposed control group. The results suggest that exposure to coal dust is associated with elevated serum BGD activity. Even in the group of ex-coalminers with normal serum LDH activity or a normal chest radiograph, a high serum BGD activity was found. All other laboratory tests were normal, which highly likely excluded the liver, heart, and muscles to be a potential source of increased serum BGD activity. Together with the knowledge that coal dust induces continuous phagocytosis and pulmonary cell damage resulting in BGD release, these results indicate that BGD activity in the studied ex-coalminers most likely originates from the lung.

The exact source of BGD activity in ex-coalminers was not directly addressed by the data in this investigation but can be speculated on. Beta-glucuronidase is known to be a membrane bound lysosomal enzyme, necessary in the hydrolysis of glucuronides, localized in the endoplasmic reticulum and in lysosomes (26). Increased phagocytic activity of AMs and PMNs, and damage to alveolar capillary barrier are reflected by an increase of BGD and LDH activities, as well as increased protein concentrations in BALF (27,28). Several animal studies associated with pulmonary cell inflammation or damage reported elevated activity of BGD in BALF after instillation of fibrogenic and non-fibrogenic particles (7,16,20,29–34). Henderson *et al.*(32) evaluated the role of the PMNs in the inflammatory response of the lung to quartz in rats with and without depletion of blood leucocytes. Neutrophil depletion did not affect the BALF activity of BGD. These results suggest that AMs but not neutrophils are the most likely source of increased BGD activity in response to quartz (32). However, other sources—such as epithelial cells, fibroblasts, and type II pneumocytes—have to be considered. The creatine kinase—although within normal ranges—was higher in the control group than in the group of ex-coalminers, which is in line with the fact that muscles are not the potential source of the increased BGD activity in the studied ex-coalminers. Consistent with other investigators (22,23), smoking history did not correlate with lysozymal activity in the studied ex-coalminers or in the control subjects, suggesting that the increase of serum BGD was not a smoking effect.

The LDH isoenzyme pattern of the lung is characterized by proportially high LDH3 and LDH4 compared to the normal serum isoenzyme pattern (35). The LDH isoenzyme pattern of AMs in BALF resembles the LDH-isoenzyme pattern of the lung (35). Therefore, the release of LDH3 occured either from injured pulmonary parenchyme or from damaged AMs and presumably produced the observed rise in serum LDH3 activity (35,36).

In the present study, the serum BGD activity was found to be elevated, even in the group of ex-coalminers with normal serum LDH activity. This could be explained by the fact that BGD only indicates a reaction of AMs to a certain pneumotoxicant but does not reflect the effect of this reaction to the lung parenchyma. The enzyme BGD can be released from inflammatory, phagocytotic cells, already before the actual lysis of the cell (37,38). In contrast,

LDH is released only after cell death induced by various mediators, which might be responsible for coherent functional impairment (31,38). With this knowledge, it can be hypothesized that the serum BGD activity is a conceivable marker for activation of AMs induced by coal dust exposure. In contrast to serum LDH activity, no correlation was found between serum BGD activity and the studied clinical parameters, which indicates that serum BGD, at first sight, is not a marker of effect.

Coal workers' pneumoconiosis, unless following a benign course, is complicated by a chronic inflammatory response and progressive massive fibrosis, caused by prolonged exposure to coal dust. However, chronic inhalation of coal dust may also cause other respiratory effects such as emphysema, chronic bronchitis and airflow obstruction (39), which might account for the functional impairment as well. Moreover, it is tempting to speculate, as in other pulmonary disorders, that a genetic predisposition is involved which might explain the various reactions of exposed individuals. Previously, the significance of oxidative stress in the development of mineral dust-related respiratory disorders has received special attention. Since antioxidant status has also been related to obstructive disease, it was suggested that the impaired oxidant/antioxidant balance observed in coal workers may also play a role in the non-pneumoconiotic respiratory effects in these subjects (10,13). Furthermore, the character and severity of lung tissue reaction to mineral dust is not predictable (39,40). Higher cumulative dust exposure does not necessarily lead to a higher profusion score on a chest radiograph (41–43). Schins *et al.* (5) did find a difference in serum TNF-R75 between a retired group of ex-coalminers and controls, but demonstrated no relation between the severity of pneumoconiosis defined by conventional chest radiograph or by high resolution computed tomography (HRCT) and plasma levels of cytokines, like TNF-R75. In line with this, we did not find differences in BGD and LDH activity between ex-coalminers with a normal and those with an abnormal chest radiograph. Furthermore, we already found a significantly elevated serum BGD activity in the group of ex-coalminers with normal chest radiograph compared to controls indicating that the increased BGD activity might reflect the intensity of inflammation in subjects after exposure to coal dust. We realize that the results of this study should be interpreted with care. In particular, the coincidence of chronic obstructive pulmonary disorders could be evaluated more carefully.

In conclusion, in this study a significant increase in serum BGD activity was demonstrated after coal dust exposure even in those subjects with a normal serum LDH activity and normal chest radiograph. Our data add *in vivo* human evidence to the already existing animal data that BGD is of potential practical value in monitoring pulmonary inflammation caused by mineral dust. To determine the significance of BGD measurement with regard to the development or progression of CWP a longitudinal design is necessary. Additional studies should focus on the BGD activity in serum as well as in BALF to illuminate its usefulness in monitoring pulmonary inflammation in addition to other biomarkers.

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